A RESONANCE RAMAN STUDY ON Hb M IWATE ($\alpha^{87\text{His}\rightarrow\text{Tyr}}\beta)_2$, AND Hb ZÜRICH ($\alpha\beta^{63\text{His}\rightarrow\text{Arg}})_2$

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Received 10 September 1974

1. Introduction

Recently it has been shown that the Raman spectrum of human haemoglobin (Hb A) provides valuable information about the structural properties of the haem group, since the frequencies of its molecular vibrations are sensitive to changes in geometry and bonding [1–3]. One of the most interesting features of the Raman spectrum of haemoglobin is the strongest Raman line near 1370 cm^{-1}. This line has been assigned to a =C-N-stretching vibration in the pyrrole rings of the haem group [1,3]. The frequency of this vibration is considerably higher in oxygenated Hb A (1376 cm^{-1}), where the iron atom is assumed to be nearly or exactly centered in the plane of nitrogen atoms of the haem group [4] than in deoxygenated Hb A (1355 cm^{-1}) where the iron atom is displaced by 0.075 nm out of the plane [5]. In methaemoglobin the frequency of this line depends upon the ligand at the 6th coordination site of the iron. A shift to lower frequencies (from 1373 to 1369 cm^{-1}) is found when comparing OH-Hb(III), a low spin Fe(III) compound at pH 9 (assumed in plane), and H_2O-Hb(III), a high spin Fe(III) compound [4,6] at pH 6 (0.03 nm out of plane), respectively. Thus in both oxidation states of haemoglobin one finds the following correlation: The more the iron is displaced out of the plane of the haem group, the lower is the frequency of the Raman line in the region of 1370 cm^{-1}. This led to the assumption that the frequency of this Raman line, henceforth called IPD (iron position dependent), monitors the position of the iron atom relative to the porphyrin plane.

In this report we have studied the behaviour of the IPD-line in relation to a change of the state of oxidation and/or ligation of the abnormal haemoglobins Hb M Iwate and Hb Zürich.

In Hb M Iwate the proximal histidine (His F8) in the α chains is replaced by tyrosine [7,8]. In this mutant the iron of the α chains has a strong tendency to be hexaco-ordinated where the distal His E7 occupies the 6th and Tyr F8 the 5th coordination site, and the iron remains in the ferric state. Binding of external ligands to the α chains is thereby excluded, and the β chains are the only binding sites for oxygen [9]. Furthermore Hb M Iwate is frozen in the quaternary

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T structure [10,11], but nevertheless shows allosteric behaviour as indicated by a considerable β–β-interaction (n = 1.6 at pH 7.0; maximum n = 1.8 above pH 9), a Bohr effect, and a DPG-effect [12,13]. Perutz has postulated the high spin–low spin transition of the iron is the allosteric trigger. Consequently it is suggested that a transition in the quaternary structure causes a change in the spin state of the iron and a change in the spin state induces a change in the quaternary structure [14].

Two questions may thus be answered by experiments on Hb M Iwate: 1) Does the iron of the α chains sense the change of the spin state in the β chains induced by ligation, and 2) is the spin state transition of the iron in the β chains restricted by the conformationally non-active α chains and the frozen quaternary T structure, respectively?

Hb Zürich shows a replacement of the distal His E7 of the β chains by arginine [15]. The Mößbauer and magnetic susceptibility data have led to the interpretation that the iron atoms of the deoxygenated β chains are in the low spin Fe(II) state [16]. Hb Zürich undergoes an allosteric transition but with diminished co-operativity (n = 1.8) [17] as compared with Hb A. These facts seem to confirm Perutz's hypothesis. The IPD-line of the Raman spectrum of Hb Zürich should be sensitive to the difference in the spin state via the different out-of-plane displacement of the iron in the α and β chains and show any influence of low spin iron(II) of the β chains on the spin state of the iron in the neighboring α chains. Thus we expected the Raman study of Hb Zürich to provide a further test of the Perutz hypothesis.

2. Experimental

Haemoglobin M Iwate was purified as described elsewhere [11,12]. Haemoglobin Zürich was isolated according to the method described in [18]. The haemoglobins were stripped from 2,3-bisphosphoglycerate by aid of gel filtration on Sephadex-G50, equilibrated with 0.1 M NaCl [19] and dissolved in 0.2 M bis-Tris buffer pH 7.0 to a concentration of approx. 5×10^{-5} M in haem. The Raman spectra were determined under the conditions described elsewhere [3].

3. Results and discussion

The Raman spectrum of the totally oxidized Hb M Iwate at pH 7 closely resembles that of oxidized Hb A. In particular, the IPD-Raman line (see fig. 1) was found at exactly the same frequency as for met Hb A namely at 1368 cm^{-1} [3]. After reduction of the haem iron of the normal β subunits of Hb M Iwate by addition of a small amount of sodium dithionite, the $(\alpha^{Mmet}\beta^{deoxy})_2$ hybrid is observed. The existence of these hybrids is proved by a characteristic visible absorption of the α^{Mmet}-chains at 610 nm. The IPD-Raman line splits apart into two lines. The line at 1368 cm^{-1} has half the intensity as compared to the similar line found for totally oxidized Hb M Iwate. This line corresponds to the =C-N-stretching vibration of the abnormal α^{Mmet} chains. The other line appears at 1355 cm^{-1} and thus is in an exact agreement with that found in deoxy Hb A. This line is therefore assigned to the =C-N-stretching vibration in the normal deoxygenated β chains of Hb M Iwate. The higher intensity of the line at 1355 cm^{-1} is simply accounted for by the fact, that in Raman spectroscopy the intensity increases for lines (λ_e^{metHb} = 408 nm; $\lambda_e^{deoxyHb}$ = 430 nm) closer to the wavelength of the exciting laser light (λ_e = 488 nm). The observed spectrum of Hb M Iwate $(\alpha^{Mmet}\beta^{deoxy})_2$ corresponds

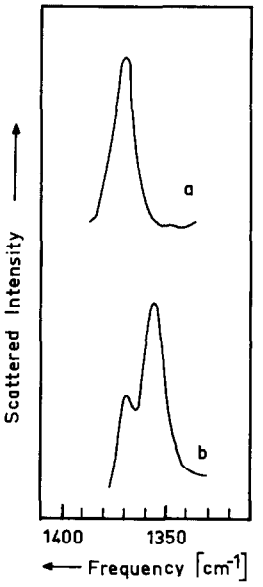

Fig. 1. Part of the resonance Raman spectra of Hb M Iwate near 1370 cm^{-1}. 5×10^{-5} M in 0.1 M bis-Tris pH 7.2. Spectral slit width: 3.2 cm^{-1}; rate of scan: 50 $cm^{-1} \times min^{-1}$; time constant: 3 s. A: totally oxidized Hb M Iwate, $\alpha_2^{Mmet}\beta_2^{met}$. B: non-oxidized Hb M Iwate, $\alpha_2^{Mmet}\beta_2^{deoxy}$.

to the combination of the Raman spectrum of Hb A in the met and deoxy form.

This simple superposition is surprising in view of the fact that the missing proximal histidine on the α chains makes a proper binding of the haem iron to the F helix impossible. One would therefore have expected a drastic alteration of the geometry in the haem cleft.

This experiment implies that upon reduction of Fe(III) (β chains) the position of the iron in the haem group of the β chains changes but the iron in the α chain haem group (Fe(III)) does not. This observation also fits with results of the electron spin resonance, which showed that ligation of β chains in Hb M Iwate with CO does not influence the symmetry of the ligand field of the haem iron of the α chains [20]. The spectral change of the haem group (IPD-line) upon reduction of the iron in the β chains of Hb M Iwate is identical to that found upon deoxygenation of Hb A. We therefore conclude that the movement of the iron relative to the haem plane (Hb A or the β chains of Hb M Iwate) and the change in spin state are similar for reduction of met forms and for the deoxygenation of Hb A. In both cases we are going to a final state of high spin Fe(II).

The absence of spectral changes for the haem groups of the α chains is consistent with the observation that the molecule remains frozen in the quaternary T state. However the observed change in β chain spectra and the changes in spin state are not consistent with Perutz's idea that in such co-operative ligand reactions the quaternary structure must change.

From our results we conclude that it is not necessary for the quaternary structure to change when the spin state changes.

In Hb Zürich the structural abnormality involves the β chain distal histidine which is replaced by arginine. Compared to Hb A its affinity for oxygen is higher, whereas it shows a smaller value for the Hill parameter (n = 1.8) [17]. In a recent publication it was concluded that, based on visible absorption, susceptibility and Mößbauer data, the iron atom in the abnormal β chains of deoxy Hb Zürich has a lower spin state than the iron in normal deoxygenated Hb A [16]. However, when Raman spectra of deoxy Hb Zürich were taken they proved nearly identical to that of normal deoxy Hb A. In particular the IPD-Raman line in deoxy Hb Zürich is exactly the same in shape and frequency as in deoxy Hb A (see fig. 2). One would expect a splitting into two lines, as in the case of Hb M Iwate, if different spin states of the two types of chains were, as expected, accompanied by different out-of-plane displacements of the iron atoms of the α and β subunits. The Raman spectrum indicates that all haem groups are equivalent with regard to their -C=N-vibrations. These results suggest that the position of the iron atom should be the same in both normal α and abnormal β chains for Hb Zürich.

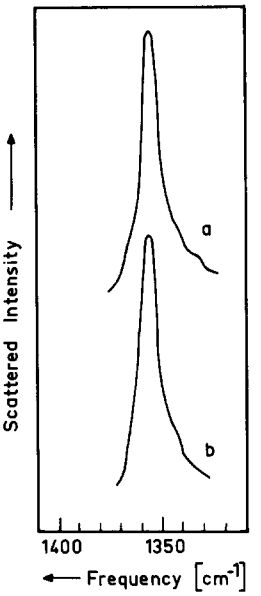

Fig. 2. Resonance Raman spectra of Hb Zürich in the region around 1370 cm^{-1}. Conditions described in the legend of fig. 1. A: deoxygenated Hb Zürich. B: deoxygenated Hb A.

Despite the large differences in O_2 affinity, co-operativity and preferred quaternary structure in Hb A, Hb M Iwate, and Hb Zürich the position of the iron atom relative to the haem plane is independent of the quaternary state of these different haemoglobins in either the non-liganded or met forms.

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